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Guignardia aesculi on species of *Aesculus*: new records from Europe and Asia

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Abstract — New localities of *Guignardia aesculi* on leaves of seven *Aesculus* species (×*carnea*, *flava*, *hippocastanum*, ×*neglecta*, *parviflora*, *pavia*, *turbinata*) were recorded in Europe. The teleomorph was found on overwintered leaves of *A. hippocastanum* in Slovakia. The occurrence of the *Guignardia* leaf blotch on *A. hippocastanum* and *A. turbinata* was also confirmed for the first time in South Korea. The causal fungus *Guignardia aesculi* and its conidial anamorph *Phyllosticta sphaeropsoidea* and spermatial synanamorph *Leptodothiorella aesculicola* are described in detail and illustrated. Pathogenicity of the fungus was confirmed by inoculating horse chestnut leaves with conidia.

Key words — *Botryosphaeriaceae*, *Hippocastanaceae*, morphology

Introduction

Guignardia aesculi (*Botryosphaeriaceae*) is a common leaf pathogen of various *Aesculus* species. It can almost totally defoliate trees through premature leaf fall. This pathogen causes serious damage of leaves (macular necrosis without leaf spot abscission) on young trees in nurseries. The symptoms (brown blotches on the leaves) can be confused with damage caused by a leaf-mining moth, *Cameraria ohridella* Deschka & Dimić. It is currently causing major problems in much of Europe, causing premature leaf fall, which looks very unattractive. However, numerous pin-point black dots (pycnidia) in the necrotic tissue distinguish blotch from insect damage and scorch due to drought or salt. Recent

invasion of a powdery mildew, *Erysiphe flexuosa* (Peck) U. Braun & S. Takam., in Europe, has been thoroughly documented (Zimmermannová-Pastirčáková et al. 2002, Kiss et al. 2004, Bolay 2005, Põldmaa 2006, Stoykov and Denchev 2008).

Guignardia aesculi is distributed in North America and Eurasia. Most of the published records include only the anamorph and the spermatial state of the fungus, but the teleomorph has been recorded in USA (Peck 1887, Stewart 1916), Western Germany (Schneider 1961), and England (Hudson 1987). The distribution of the fungus in Asia as well as the teleomorph has been very poorly studied. There are no modern detailed descriptions of all the different states of *G. aesculi* available.

This contribution deals with morphology, pathology, host range, and geographical distribution of the causal agent of horse chestnut leaf blotch.

Material and methods

Collections and microscopic observations — Living leaves of *Aesculus* ×*carnea* Hayne, *A. flava* Sol., *A. hippocastanum* L., *A.* ×*neglecta* Lindl., *A. parviflora* Walter, *A. pavia* L. and *A. turbinata* Blume naturally infected with the anamorph of *Guignardia aesculi* were collected in 2000–08 in ten European countries (Austria, Belgium, Croatia, Czech Republic, Hungary, The Netherlands, Poland, Russia, Slovakia, Slovenia), USA, and South Korea. Regular collecting of overwintered leaves was conducted to find the teleomorph of the fungus. In castle park in Ivanka pri Dunaji, Slovakia, a number of *A. hippocastanum* trees naturally infected with *G. aesculi* were marked in the autumn of 2000. The fallen infected leaves of *A. hippocastanum* lying on the ground were collected every 14 days in March and April 2001. Sampling was repeated in March and April 2008 in four Slovak localities (Bratislava, Giraltovce, Leopoldov, Nitra).

All samples were examined by a stereo (SZ51, Olympus, Tokyo, Japan) and a compound microscope (BX51, Olympus, Tokyo, Japan). Slide preparations were stained with lactophenol blue solution (Merck, Darmstadt, Germany). The observed characters were compared to previously published descriptions and documented photographically along with symptoms of attack.

Herbarium specimens were deposited in the herbarium of Institute of Forest Ecology SAS, Nitra, Slovakia. Selected specimens were deposited in the mycological herbarium of Slovak National Museum, Bratislava, Slovakia (BRA) and at the U.S. National Fungus Collections, USA (BPI). Collections from Slovenia were deposited in the private herbarium of F. Celar.

Isolation and inoculation — The fungus was isolated from samples of infected leaves of *A. hippocastanum* collected in Kračúnovce, Slovakia, in July 2000. Leaf tissue pieces with lesions were surface sterilized with 0.5% NaOCl for 1 min. Small pieces of tissue were excised, transferred to Petri dishes containing malt extract agar (Merck, Darmstadt, Germany), and incubated at 23±2°C. Colonies growing from the tissue pieces were subcultured onto malt extract agar and rice agar after five days. Test cultures were incubated on plates for two weeks.

To confirm the fungus pathogenicity and fulfill Koch's postulates, 3-yr-old seedlings of *A. hippocastanum* were inoculated with a *Phyllosticta* isolate. Healthy leaves of ten seedlings growing in plant pots in greenhouse were sprayed with the conidial suspension of the *Phyllosticta* anamorph of 2×10^5 conidia/ml to runoff with a hand-held atomizer. The inoculated leaves were covered with test tubes and the opening of the tubes plugged with cotton for four days. Five non-inoculated seedlings were used as a control and maintained in the same conditions in another compartment in greenhouse.

Taxonomic description

Guignardia aesculi (Peck) V.B. Stewart, Phytopathology 6: 9, 1916
≡ *Laestadia aesculi* Peck, Ann. Rep. N.Y. St. Mus. Nat. Hist. 39: 51, 1887
≡ *Botryosphaeria aesculi* (Peck) M.E. Barr, Contr. Univ. Mich. Herb. 2: 561, 1972

Anamorph: *Phyllosticta sphaeropsoidea* Ellis & Everh., Bull. Torrey Bot. Club 10: 97, 1883
≡ *Phyllostictina sphaeropsoidea* (Ellis & Everh.) Petr., Sydowia 10: 265, 1957
?= *Phyllosticta paviae* Desm., Ann. Sci. Nat., Bot., sér. 3, 8: 32, 1847

Synanamorph: *Leptodothiorella aesculicola* (Sacc.) Sivan., Bitunic. Ascomyc.: 165, 1984
≡ *Phyllosticta aesculicola* Sacc., Michelia 1: 134, 1878
≡ *Asteromella aesculicola* (Sacc.) Petr., Sydowia 10: 266, 1957
= *Phyllosticta aesculi* Ellis & G. Martin, J. Mycol. 2: 130, 1886

Representative specimens examined — On leaves of *Aesculus ×carnea*, **AUSTRIA:** Tulln, 18 Oct 2001, leg. K. Pastirčáková (BRA CR9831); Vienna, city centre, 12 Sep 2002, leg. M. Pastirčák (BPI 871186); **CZECH REPUBLIC:** Prague, Mala Strana, 10 Sep 2002, leg. K. Pastirčáková; **HUNGARY:** Budapest, Orlay utca, Várkert Rakpart, 19 Aug 2006, leg. K. Pastirčáková; **SLOVAKIA:** Rimavská Sobota, city park, 24 Aug 2001, leg. K. Pastirčáková (BPI 871185); **SLOVENIA:** Ljubljana, Rožna dolina, near old Tobacco factory, Sep 2002, leg. F. Celar.

On leaves of *Aesculus flava*, **SLOVENIA:** Ljubljana, near National Institute of Biology, May 2008, leg. F. Celar.

On leaves of *Aesculus hippocastanum*, **AUSTRIA:** Tulln, 22 Sep 2001, leg. K. Pastirčáková (BRA CR9832); Tulln, near Institute for Agrobiotechnology, 18 Oct 2001, leg. M. Pastirčák (BPI 871183); **BELGIUM:** Plankendaal, 10 Aug 2001, leg. M. Lemmens; **CROATIA:** Zagreb, Cmrok, park, 20 Sep 2001, leg. D. Diminić; **CZECH REPUBLIC:** Prague, city centre, 10 Sep 2002, leg. K. Pastirčáková (BPI 871181); **HUNGARY:** Budapest, Döbrentei tér, Várkert Rakpart, 19 Aug 2006, leg. K. Pastirčáková; **THE NETHERLANDS:** Wageningen, 10 Oct 2001, leg. A.M. de Haas; **POLAND:** Lublin, park, 9 Nov 2000, leg. K. Pastirčáková; **RUSSIA:** Saint-Petersburg, Ploschad Chenyshevskogo, 22 Sep 2007, leg. K. Pastirčáková; **SLOVAKIA:** Kračúnovce, school park, 28 Jul 2000, leg. K. Pastirčáková (BRA CR9833); Ivanka pri Dunaji, castle park, 21 Sep 2000, leg. K. Pastirčáková; Giraltovce, city park, 20 Aug 2001, leg. K. Pastirčáková (BPI 871184); Bratislava, Rusovce, park, Mar–Apr 2008, leg. M. Pastirčák; Giraltovce, city park, Mar–Apr 2008, leg. M. Pastirčák; Leopoldov, city, Mar–Apr 2008, leg. M. Pastirčák; Nitra, Nábrežie mládeže, Mar–Apr 2008, leg. K. Pastirčáková; **SLOVENIA:** Ljubljana, Rožna dolina, near old Tobacco factory, Sep 2002, leg. F. Celar; **SOUTH KOREA:** National Arboretum, 30 Sep 2005, leg. K. Pastirčáková; **USA:** Grove City, Pennsylvania, 14 Oct 2000, leg. P.M. Glova.

On leaves of *Aesculus ×neglecta*, **SLOVENIA:** Arboretum Volčji Potok, Sep 2006, leg. F. Celar.

On leaves of *Aesculus parviflora*, **AUSTRIA:** Vienna, city park, 26 Oct 2001, leg. K. Pastirčáková; **SLOVAKIA:** Arboretum Mlyňany, 21 Jul 2001, leg. K. Pastirčáková; **SLOVENIA:** Ljubljana, Botanical Garden, Sep 2006, leg. F. Celar.

On leaves of *Aesculus pavia*, **SLOVENIA:** Ljubljana, near National Institute of Biology, May 2008, leg. F. Celar.

On leaves of *Aesculus turbinata*, **SLOVENIA:** Ljubljana, near Biotechnical faculty, Aug 2007, leg. F. Celar; **SOUTH KOREA:** Forest Practice Research Center KFRI, 30 Sep 2005, leg. K. Pastirčáková; Seoul, Gwanghwamun, 5 Oct 2005, leg. K. Pastirčáková.

Symptoms — The first symptoms of leaf blotch disease, water-soaked irregular areas on living leaves, appear in May. Within a few days these turn reddish to brown, obtain a yellow border and coalesce. The lesions are variable in size and shape, often concrescent and cover extensive areas of the leaf. Minute black specks are seen scattered over the lesion (Figure 1A). Large lesions cause curling and distortion of leaflets. Small reddish brown elongate spots on petioles are also present.

Microscopic features — Teleomorph (based on Slovak material): Pseudothecia on dead leaves in spring, subepidermal, globose to subglobose (Figure 2A), solitary, dark brown, wall of textura angularis with thick-walled cells of outer layers (Figure 2B), ostiolate, 90–180 µm in diameter, containing 20–26 asci. Asci bitunicate, clavate to cylindrical, stalked, thickened and rounded at the apex, 8-spored, 55–80 × 16–28 µm (Figures 2C–2F). Ascospores one-celled, hyaline, straight or slightly curved, ovoid, ellipsoidal or rhomboidal, with a granular content, rarely guttulate, usually wider in the middle, 14–18 × 7–9 µm (Figures 2G–2I). Young ascospores possess mucilaginous cap-shaped appendages at one or both ends, which disappear at maturity. Pseudoparaphyses absent in mature pseudothecia.

Anamorph: Pycnidia on leaf spots in living leaves in summer and autumn, on both surfaces of lesions, but much more on the upper surface, epiphyllous, single, globose to pyriform, dark brown, immersed, 80–160 µm in diameter, unilocular, ostiolate, wall of textura angularis (Figures 1B, 3A). Subcircular ostiole 10–18 µm in diameter, surrounded by conspicuous dark brown cells. Conidiogenous cells holoblastic, cylindrical or conical, hyaline. Conidia hyaline, coarsely granular, rarely guttulate, smooth, thin-walled, one-celled, globose, ellipsoidal, clavate or obpyriform, 9–18 × 6–12 µm, surrounded by a thin mucilaginous sheath, with a hyaline apical appendage, 6–8(–12) µm long (Figures 3B–3F). Conidia held together by the gelatinous substance seen extruding from the ostiole as a white coil.

Synanamorph: Spermogonia similar in morphology to pycnidia, immersed, globose, dark brown, 40–95 µm in diameter. Spermatiogenous cells holoblastic, filamentous to cylindrical, long and slender. Spermatia one-celled, oblong cylindrical to dumb-bell shaped, hyaline, guttulate, straight or slightly curved, 3–10 × 0.5–2.5 µm (Figure 3G).

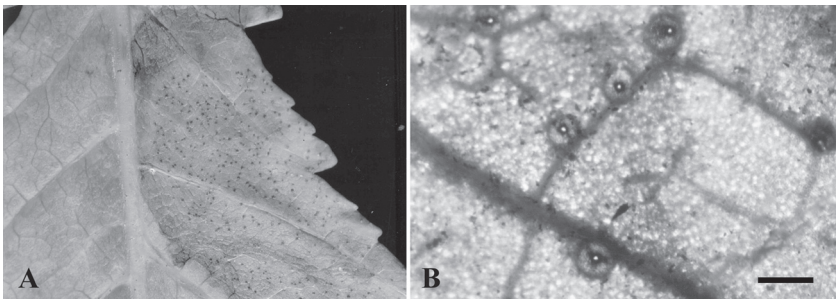

Figure 1. *Guignardia* leaf blotch. (A) a blotch with numerous pycnidia on naturally infected leaf of *Aesculus hippocastanum*; (B) pycnidia of the *Phyllosticta* anamorph in the necrotic tissue as viewed with a hand lens. Scale bar = 200 μm.

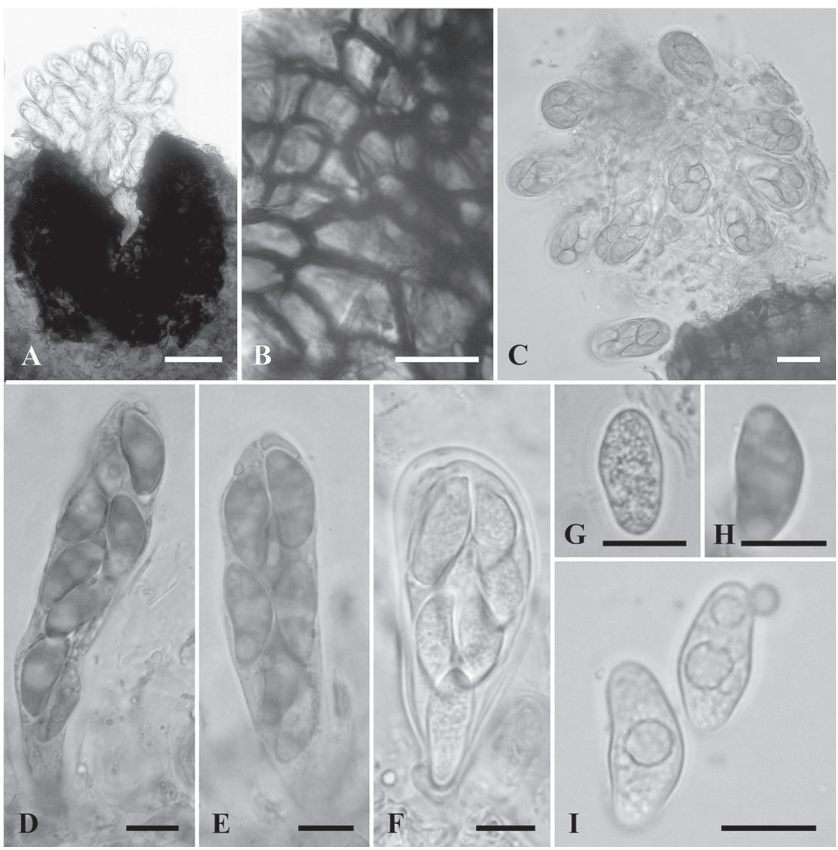

Figure 2. *Guignardia aesculi*. (A) pseudothecium with asci; (B) wall of pseudothecium of textura angularis with thick-walled cells of outer layers; (C) a cluster of mature asci; (D–F) bitunicate asci and ascospores; (G–I) ascospores. Scale bars = 40 μm (A), 20 μm (B), 30 μm (C), 10 μm (D–I).

Colonies: Isolates produced different colonies on malt extract agar and rice agar (Figure 4). Colonies grew slowly, on malt extract agar: aerial mycelium woolly, white, hyphae branched, septate, hyaline, 4–6 μm in wide (Figure 3H), dark pycnidia clustered, aggregated; on rice agar: aerial mycelium scanty, pycnidia abundant, dark, globose, solitary, rarely clustered, with conidial and spermatial cavities; ascomata not produced in culture. Cultural characters and the effect of different nutrient media, their pH value and the ultraviolet radiation on mycelial growth and sporulation in isolates of *P. sphaeropsoidea* have been described in an earlier paper (Zimmermannová-Pastirčáková 2003).

The fungus was identified as *Guignardia aesculi* on the basis of the characteristics described above. The morphological features of the teleomorph, anamorph and spermatial state observed in the collections from seven species of *Aesculus* (*A.* ×*carnea* [*hippocastanum* × *pavia*], *A. flava*, *A. hippocastanum*, *A.* ×*neglecta* [*flava* × *sylvatica*], *A. parviflora*, *A. pavia* and *A. turbinata*) correspond to those reported in previous publications (Stewart 1916, Sivanesan 1984, Punithalingam 1993).

The pycnidial anamorph was found in all examined samples. The spermatial state was found in most of the specimens, except for those of *A. hippocastanum* collected in Croatia and Czech Republic. Spermatia can be found in the same pycnidium together with *Phyllosticta* conidia (Treigiene 2006). A spermogonium has frequently been reported to develop immediately next to and in the same stroma with pycnidium, the two being separated only by a thin membranous wall (Stewart 1916).

The teleomorph was recorded on *A. hippocastanum* in Slovakia. The sexual fruiting bodies of the examined fungus were not present from June to November when the leaves were collected. The fungus forms pseudothecia in the old lesions during the winter and mature in the spring when new leaves are developing. Overwintering and formation of teleomorph of the fungus was repeatedly observed on naturally infected leaves of *A. hippocastanum* in 2001 and 2008 in four localities in Slovakia. The mature pseudothecia with asci and differentiated ascospores were observed in Slovak specimens collected in April 2001. In 2008 numerous mature pseudothecia were seen in lesions of specimens collected as early as 30th March. The description of the teleomorph based on these collections is given above.

Pathogenicity — Typical symptoms of leaf blotch began to develop approximately 10 days after the inoculation of *A. hippocastanum* seedlings with conidia. The symptoms of leaves artificially inoculated were identical to those of naturally infected leaves in the field. Further, pycnidia with conidia appeared in the lesions after 7 days. Control plants remained healthy. The pathogenicity test revealed that the isolate of *Phyllosticta sphaeropsoidea* from leaves of *A. hippocastanum* from Slovakia was pathogenic and when re-isolated from

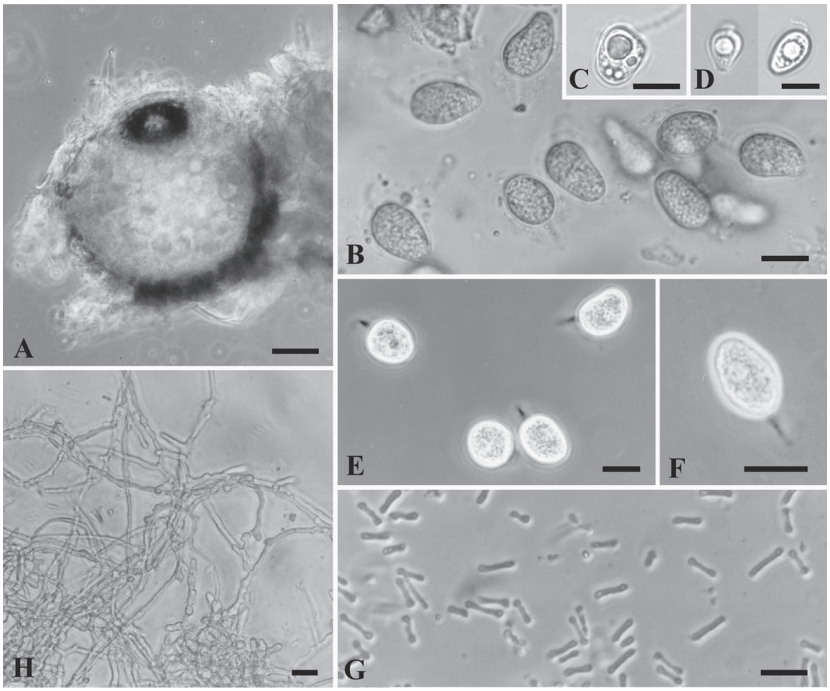

Figure 3. The anamorphs of *Guignardia aesculi*. (A–F) *Phyllosticta* anamorph: (A) pycnidium; (B, E, F) conidia with a granular content and (C, D) conidia with guttulae and apical appendages. (G, H) *Leptodothiorella* synanamorph: (G) spermatia; (H) hyphae of anamorphic state in culture. Scale bars = 30 μm (A), 10 μm (B–G), 20 μm (H).

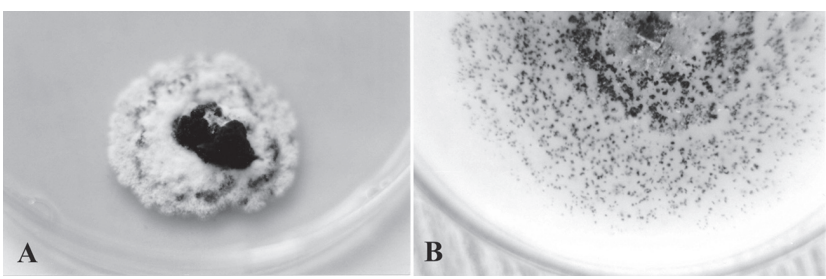

Figure 4. Cultures of *Phyllosticta* anamorph of *Guignardia aesculi*, 14-d old, inoculated from leaves of *Aesculus hippocastanum*. (A) on malt extract agar, (B) on rice agar.

successfully infected leaves, the fungus was identical to the initial isolate. Only *A. hippocastanum* seedlings were used for pathogenicity test because seedlings of other species could not be obtained.

Hudson (1987) reported characteristic lesions on horse chestnut leaves 4–6 weeks after inoculation with separate suspensions of conidia and ascospores.

Table 1. Global distribution of *Aesculus* species attacked by *Guignardia aesculi*

Geographic region		*Aesculus* spp.	References
North America	Canada	*glabra*, *hippocastanum*	Bissett & Darbyshire 1984
	USA	×*ambigua*, *arguta*, ×*arnoldiana*, ×*bushii*, *californica*, ×*carnea*, *chinensis*, *discolor*, *flava*, *georgiana*, *glabra*, ×*glaucescens*, *hippocastanum*, ×*hybrida*, ×*mutabilis*, ×*neglecta*, *parviflora*, *pavia*, *splendens*, *turbinata*, ×*woerlitzensis*	Neely & Himelick 1963, Neely 1971, Farr et al. n.d.
Europe	Austria	×*carnea*, *hippocastanum*, *parviflora*	Petrak 1957, this paper
	Bulgaria, Croatia, Estonia, Italy, Lithuania, Norway, Poland, Romania, Russia, Ukraine	*hippocastanum*	Scaramuzzi 1954, Milatović 1956, van der Aa 1973, Fakirova 1993, Andrianova 2006, Põldmaa 2006, Talgø et al. 2006, Treigiene 2006, this paper
	Belgium, England	*hippocastanum*, *indica*, *parviflora*	Grove 1935, Hudson 1987, Farr et al. n.d.
	Czech Republic, Hungary, Portugal, Switzerland	×*carnea*, *hippocastanum*	Scaramuzzi 1954, Caetano 1985, Bolay 2000, Magyar & Tóth 2003, this paper
	France	×*carnea*, *hippocastanum*, *indica*, *parviflora*	Vegh & Le Berre 1991, Farr et al. n.d.
	Germany	*glabra*, *hippocastanum*	Schneider 1961, Farr et al. n.d.
	Netherlands	*californica*, ×*carnea*, *hippocastanum*, *indica*, ×*mutabilis*, ×*neglecta*, *pavia*, *sylvatica*, ×*woerlitzensis*	de Haas pers. comm. 2001
	Slovakia	×*carnea*, *hippocastanum*, *parviflora*, *pavia*	Hrubík 1976, Juhásová et al. 1998, Farr et al. n.d., this paper
	Slovenia	×*carnea*, *flava*, *hippocastanum*, ×*neglecta*, *parviflora*, *pavia*, *turbinata*	Milatović 1956, this paper
Asia	Armenia	*hippocastanum*	Simonyan 1981
	China	*pavia*	Chen et al. 2007
	South Korea	*hippocastanum, turbinata*	This paper

Stewart (1916) carried out successful cross inoculations of *A. glabra* Willd. and *A. hippocastanum* seedlings by ascospores, with incubation period from 10 to 20 days, whereas inoculations of *A. parviflora* leaves with ascospores from *A. hippocastanum* failed. According to van der Aa (1973), inoculation experiments with conidia and ascospores of species of *Guignardia* have mostly shown that these are specific plant pathogens capable of infecting one host species or species of one host genus.

Host range and distribution — The host range of *G. aesculi* is restricted to representatives from family *Hippocastanaceae*. Records of *Phyllosticta paviae* on

Thuja occidentalis L. and *Hamamelis* sp. in USA (Farr et al. n.d.) are probably assignable to *P. thujae* Bissett & M.E. Palm and *P. hamamelidis* Cooke ex G. Martin, respectively. Table 1 provides a list of all species and hybrids of the genus *Aesculus* attacked by *G. aesculi* reported in North American, European and Asian countries based on data in the literature. There are no herbarium collections of this fungus on the Asiatic species *A. assamica* Griff. and *A. parryi* A. Gray and the Mexican species *A. wilsonii* Rehder deposited in mycological herbaria listed in the Index Herbariorum database, and no records on these hosts in the literature.

For the first time we recorded *Guignardia* leaf blotch on *A.* ×*carnea* in Austria, Czech Republic and Hungary; on *A. hippocastanum* in Russia; and on *A. parviflora* in Austria and Slovakia. Six *Aesculus* species (*A.* ×*carnea*, *A. flava*, *A.* ×*neglecta*, *A. parviflora*, *A. pavia*, *A. turbinata*) are reported as new hosts for *G. aesculi* in Slovenia. The specimens from South Korea represent the first record of the species in the country and the third known locality from Asia.

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